REMARKS

This paper is filed in response to the final official action dated August 1, 2007. This paper is timely-filed.

Claims 1-12, 14-16, 18-19, and 21 are pending. By the foregoing, claims 1, 3-5, and 18 have been amended to correct typographical errors. No new matter has been added.

Additionally, all pending claims 1-12, 14-16, 18-19, and 21 have been rejected under 35 U.S.C. §103(a) as obvious over Fitzpatrick *et al.* in view of Kim *et al.*

The sole basis for the claim rejections is addressed below. Reconsideration of the application as amended is solicited in view of the following remarks.

CLAIM REJECTIONS -- 35 U.S.C. §103(a)

The applicants respectfully traverse the rejections of all pending claims 1-12, 14-16, 18, 19, and 21 as obvious over Fitzpatrick *et al.* in view of Kim *et al.*

The examiner suggested that the applicants attacked the references individually in their last response. Reconsideration is respectfully requested.

The applicants previously argued that Kim *et al.* generally teaches that racemization metal catalysts must be compatible with both the acyl donors and the protein hydrolysis enzymes in the reaction system. *See* Kim *et al.* at pages 579 and 581. The applicants also pointed out that Kim *et al.* does not disclose or suggest that racemization metal catalysts of Fitzpatrick *et al.* are compatible with the protein hydrolysis enzymes recited by claims 1 and 18, and therefore submitted that all pending claims are patentable over Fitzpatrick *et al.* and Kim *et al.*, whether taken alone or *in combination*.

Essentially, the applicants submitted that the combination of Fitzpatrick *et al.* and Kim *et al.* does not provide a reasonable expectation of success for the proposed combination. In further support of applicants' assertion that Fitzpatrick *et al.* and Kim *et al.* do not provide a reasonable expectation of success for the proposed combination, the applicants respectfully submit that Fitzpatrick *et al.* also teaches that "[a] major obstacle to a wider exploitation of enzyme selectivity is its *relative inflexibility*." *See* Fitzpatrick *et al.* at page 3166.

A reasonable expectation of success is necessary to sustain a *prima facie* case of obviousness. The applicants respectfully submit that a reasonable expectation of success cannot be established for the proposed combination, particularly in view of the well-documented sensitivity of enantioselective reaction systems. Accordingly, the rejections of claims 1-12, 14-16, 18, 19, and 21 as obvious over Fitzpatrick *et al.* in view of Kim *et al.* should be withdrawn.

Furthermore, claim 1 recites "stabilized subtilisin" whereas Fitzpatrick uses subtilisin itself. Therefore, the proposed modification would not result in the claimed invention and the examiner has not demonstrated all of the elements recited in claim 1 as required to sustain a *prima facie* case of obviousness.

Additionally, prior to the applicants' invention subtilisin itself was generally known to have poor activity, selectivity, and stability in organic solvents, and therefore one of ordinary skill would not have a reasonable expectation of success in using it in dynamic kinetic resolution in organic solvent. In support of this assertion, attached hereto as Attachment A is applicants' paper (Kim *et al.*, JACS, *125:*11494-11495, August 30, 2003). The applicants direct the examiner to the second full paragraph thereof.

CONCLUSION

It is respectfully submitted that this application is now in condition for allowance. Should the examiner wish to discuss the foregoing, or any matter of form or procedure in an effort to advance this application to allowance, she is respectfully invited to contact the undersigned attorney at the indicated telephone number.

Respectfully submitted,

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COMMUNICATIONS

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(*S*)-Selective Dynamic Kinetic Resolution of Secondary Alcohols by the Combination of Subtilisin and an Aminocyclopentadienylruthenium Complex as the Catalysts

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Dynamic kinetic resolution (DKR) provides a useful methodology for the conversion of racemic substrates to single enantiomeric products.[1] Recently, several groups, including ours, have reported the use of enzyme−metal combinations as the catalyst system for DKR.[2] In the DKR, a metal complex acts as a racemizing catalyst, and an enzyme, as a resolving catalyst. As the result, a racemic mixture transforms to an enantiomerically enriched product. All the enzyme/metal-catalyzed DKR reactions reported thus far have employed a lipase as the resolving catalyst.[3−5] Accordingly, only the products of (*R*)-configuration are available in case simple secondary alcohols are resolved. We herein wish to report for the first time a complementary procedure using subtilisin as the resolving catalyst and an aminocyclopentadienylruthenium complex as the racemizing catalyst for the products of (*S*)-configuration.

In general, subtilisin is inferior to lipase in activity, selectivity, and stability for nonaqueous biocatalysis.[6] As a result, the use of subtilisin in kinetic resolution (KR) has been thus far limited to a few applications, while lipases have found wide applications. We, however, thought that the utility of subtilisin would be expanded by its use in DKR. In principle, DKR provides higher enantiomeric excess (ee) and better yield than the corresponding KR. In addition, subtilisin-based DKRs should provide a complementary stereoselectivity to the lipase-based DKRs since the stereospecificity of subtilisin is opposite to that of lipase.[6]

A subtilisin-based DKR of 1-phenylethanol (**1a**) is described as the route **A** in Scheme 1. The (*S*)-selective acylation of **1a** by subtilisin in the presence of an acyl donor takes place with the racemization of **1a**, catalyzed by the ruthenium complex **3**.[7] On the other hand, the (*R*)-selective acylation can be achieved with lipase as shown in the route **B**. We have already reported that **3** is highly active and the (*R*)-selective DKR proceeds efficiently at room temperature.[4f]

Subtilisins usually exhibit low activity in organic solvents. For instance, a commercial subtilisin (*Bacillus licheniformis*, lyophilized powder)[8] showed 0.8 nmol/mg of enzyme/h of activity in the acylation of **1a** with vinyl butyrate in THF. In preliminary studies, the DKR of **1a** with the commercial subtilisin was unsuccessful due to the low activity. To enhance the activity, the enzyme was treated with a nonionic surfactant, polyoxyethylene(10) cetyl ether[8] ($C_{16}H_{33}(OCH_2CH_2)_nOH$, $n = \sim 10$; trade name, Brij 56) before use.[9] The surfactant treatment resulted in a dramatic increase of the enzyme activity in THF. It was observed that the surfactant-treated subtilisin (STS) was 3 orders of magnitude (about 4000 times) more active than its untreated counterpart. Furthermore, the surfactant treatment significantly enhanced the enzyme stability. STS displayed 70% of its original activity after 5 days incubation in THF at 25 °C, while the commercial enzymes in lyophilized powder or cross-linked crystals (CLEC) almost completely lost the activities after 5 days.[10]

Scheme 1. Dynamic Kinetic Resolution of 1-Phenylethanol

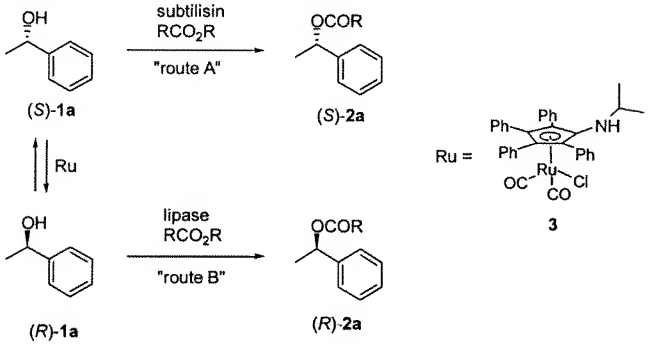

Table 1. Dynamic Kinetic Resolution of 1-Phenylethanol

entry	solvent	acyl donor[a]	mol % of **3**	% yield[b]	% ee[c]
1	2,2,4-trimethylpentane	PCPB	10	88	80
2	toluene	PCPB	10	86	79
3	*t*-butyl methyl ether	PCPB	10	93	82
4	methylene chloride	PCPB	10	91	87
5	1,4-dioxane	PCPB	10	98	84
6	*tert*-butanol	PCPB	10	94	91
7	THF	PCPB	10	98	89
8	THF	IPA	10	22	71
9	THF	TFEA	10	60	52
10	THF	TFEB	10	93	88
11	THF	TFEB	8	95	91
12	THF	TFEB	6	93	88
13	THF	TFEB	4	95	92
14	THF	TFEB	2	82	70

[a] PCPB, *p*-chlorophenyl butyrate; IPA, isopropenyl acetate; TFEA, trifluoroethyl acetate; TFEB, trifluoroethyl butyrate. [b] Determined by ^{1}H NMR. [c] Measured by chiral HPLC (Whelk-O1).

The DKR of **1a** with STS was tested on 0.3 mmol scale with varying the acyl donor, the amount of **3**, and the solvent at 25 °C for 3 days. First, the DKR was examined with STS (60 mg/mmol substrate) and 10 mol % of **3**[7] in the presence of *p*-chlorophenyl butyrate (PCPB) as an acyl donor in seven different solvents (entries 1−7, Table 1). The highest yield (98%) was obtained in THF and 1,4-dioxane (entries 5 and 7), and the highest ee's (89−91% ee) were obtained in THF and *tert*-butyl alcohol (entries 6−7). Both the yield and the ee were relatively lower in hydrophobic solvents such as 2,2,4-trimethylpentane and toluene. Second, the DKR was carried out with varying the acyl donor in THF (entries 7−10). The DKR with PCPB showed the best efficiency, in which the yield and the ee reached 98% and 89%, respectively (entry 7). A similar result (93% yield and 88% ee) was obtained with a less reactive acyl donor, TFEB (entry 10). Trifluoroethyl acetate (TFEA) was not satisfactory, and isopropenyl acetate (IPA) was practically inefficient. Finally, the DKR was performed with varying the amount of **3** in the presence of TFEB in THF (entries 10−14). No

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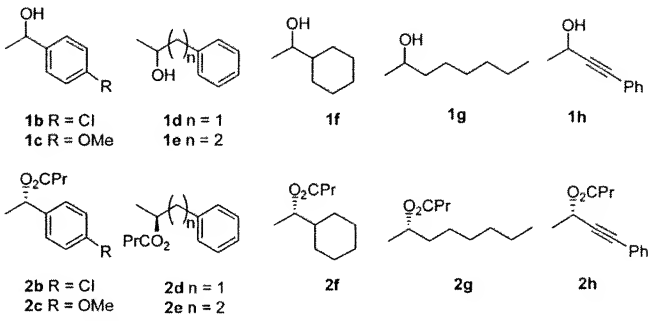
ATTACHMENT A

Table 2. Dynamic Kinetic Resolution of Secondary Alcohols by Subtilisin–Ruthenium Combination



entry	substrate	product	mol % of **3**	% yield[a]	% ee
1	**1b**	**2b**	4	92(90)	99[b]
2	**1c**	**2c**	4	93(91)	94[b]
3	**1d**	**2d**	4[d]	77(76)	97[b]
4	**1d**	**2d**	10[e]	89	92[b]
5	**1e**	**2e**	4[d]	80(78)	98[b]
6	**1e**	**2e**	10[e]	95	98[b]
7	**1f**	**2f**	4[d]	80(74)	98[c]
8	**1f**	**2f**	10[e]	92	98[c]
9	**1g**	**2g**	4[d]	77(67)	98[c]
10	**1g**	**2g**	10[e]	89	98[c]
11	**1h**	**2h**	4	90(90)	95[b]

[a] By ^{1}H NMR. The isolated yield is given in parentheses. [b] By HPLC (Whelk-O1). [c] By GC (Chiraldex B–PH). [d] Condition **A**: 25 mg of STS/mmol substrate and 4 mol % of **3**. [e] Condition **B**: 60 mg of STS/mmol substrate and 10 mol % of **3**.

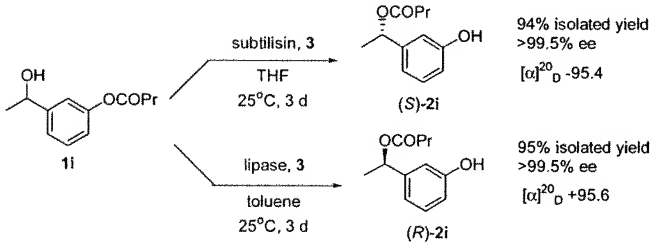
significant difference in yield and ee was observed in the range of 10 to 4 mol % (entries 10–13), but 2 mol % was too small to give an acceptable result (entry 14).

On the basis of the above results, THF was chosen as the solvent for the DKR of other substrates **1b–h** to see the scope of our catalyst system (Table 2). TFEB was chosen as the acyl donor, because the isolation of products was easier with TFEB than with PCPB. The DKRs (0.3 mmol scale) of **1b**, **1c**, and **1h** were carried out with STS (25 mg/mmol substrate) and 4 mol % of **3** in THF at 25 °C for 3–4 days. For unactivated alcohols **1d–g**, their DKRs were examined under two different conditions (condition A, 25 mg of STS/mmol substrate and 4 mol % of **3**; condition B, 60 mg of STS/mmol substrate and 10 mol % of **3**).

p-Chlorophenyl methyl carbinol (**1b**) was transformed in a high yield (92%) with a high ee value (99%). The excellent ee reflects the high enantioselectivity ($E \geq 400$) of STS toward **1b**, which was confirmed by a separate kinetic resolution experiment. *p*-Methoxyphenyl methyl carbinol (**1c**) and phenylalkynyl methyl carbinol (**1h**) were transformed also in high yields (90–93%), but with slightly lower ee values (94–95%). The condition A for the DKR of **1d–g** gave high ee values (97–98%) but low yields (77–80%). The yields, however, increased up to 95% under the condition B, employing 2.5-fold more enzyme and metal catalyst. These results reflect the relatively slow racemization of the aliphatic substrates.[11]

To show that the subtilisin-catalyzed DKR is complementary to its lipase-catalyzed counterpart, both DKRs were performed with **1i** at 25 °C (Scheme 2). Here, no acyl donor was added because the substrate itself carries an acyl group. Both DKRs afforded high ee values and excellent yields. As expected, the product from the subtilisin-catalyzed DKR showed an optical rotation opposite that from the lipase-catalyzed DKR.

Scheme 2. Dynamic Kinetic Resolution of *m*-Butanoyloxyphenyl-1-ethanol



We have demonstrated that the (*S*)-selective DKR of alcohols has been successfully achieved by the combination of subtilisin and an aminocyclopentadienylruthenium complex. The success of the DKR is based on three important factors: the enhanced activity and stability of surfactant-treated subtilisin, the high activity of the ruthenium complex at room temperature,[4f] and the good compatibility between these two catalysts. It is now possible to transform a wide range of racemic alcohols into their acyl derivatives enantioselectively through a pair of complementary DKRs. The methodology should find use, particularly in the synthesis of chiral drugs and their building blocks.

Acknowledgment. This work was supported by the Korean Ministry of Science and Technology (NRL program) and POSCO. We thank the Korean Ministry of Education (BK21 program) for its support to our graduate program.

Supporting Information Available: General DKR procedure and analytical data (PDF). This material is available free of charge via the Internet at http://pubs.ac.org.

References

(1) (a) Ward, R. S. *Tetrahedron: Asymmetry* **1995**, *6*, 1475.
(2) Reviews: (a) Huerta, F. F.; Minidis, A. B. E.; Bäckvall, J.-E. *Chem. Soc. Rev.* **2001**, *30*, 321. (b) Kim, M.-J.; Ahn, Y.; Park, J. *Curr. Opin. Biotechnol.* **2002**, *13*, 578. (c) Pàmies, O.; Bäckvall, J.-E. *Chem. Rev.* **2003**, *103*, 3247.
(3) (a) Persson, B. A.; Larsson, A. L. E.; Ray, M. L.; Bäckvall, J.-E. *J. Am. Chem. Soc.* **1999**, *121*, 1645. (b) Huerta, F. F.; Laxmi, Y. R. S.; Bäckvall, J.-E. *Org. Lett.* **2000**, *2*, 1037. (c) Pamies, O.; Bäckvall, J.-E. *J. Org. Chem.* **2001**, *66*, 4022. (d) Pamies, O.; Bäckvall, J.-E. *J. Org. Chem.* **2002**, *67*, 1261. (e) Dijksman, A.; Elzinga, J. M.; Li, Y.-X.; Arends, I. W. C. E.; Sheldon. R. A. *Tetrahedron: Asymmetry* **2002**, *13*, 879.
(4) (a) Koh, J. H.; Jung, H. M.; Kim, M.-J.; Park, J. *Tetrahedron Lett.* **1999**, *40*, 6281. (b) Jung, H. M.; Koh, J. H.; Kim, M.-J.; Park, J. *Org. Lett.* **2000**, *2*, 409. (c) Lee, D.; Huh, E. A.; Kim, M.-J.; Jung, H. M.; Koh, J. H.; Park, J. W. *Org. Lett.* **2000**, *2*, 2377. (d) Jung, H. M.; Koh, J. H.; Kim, M.-J.; Park, J. *Org. Lett.* **2000**, *2*, 2487. (e) Kim, M.-J.; Choi, Y. K.; Choi, M. Y.; Kim, M. J.; Park, J. *J. Org. Chem.* **2001**, *66*, 4736. (f) Choi, J. H.; Kim, Y. H.; Nam, S. H.; Shin, S. T.; Kim, M.-J.; Park, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 1037.
(5) (a) Reetz, M. T.; Schimossek, K. *Chimia* **1996**, *50*, 668. (b) Choi, Y. K.; Kim, M. J.; Ahn, Y.; Kim, M.-J. *Org. Lett.* **2001**, *3*, 4099.
(6) Kazlauskas, R. J.; Weissfloch, A. N. E. *J. Mol. Catal. B: Enzym.* **1997**, *3*, 65.
(7) Ruthenium complex **3** is activated by the treatment with potassium *tert*-butoxide just before use (see ref 4f). It is now available from Strem.
(8) It was purchased from Sigma.
(9) The procedure for the preparation of STS: Brij 56 (1.17 g, 1.72 mmol) in aqueous pyridine (2.3% H_2O, 4.4 mL) was sonicated for 5 min, followed by the addition of subtilisin (40 mg). The resulting mixture was stirred for 12 h at 35 °C and then centrifuged to isolate undissolved solid. The solid was dried in vacuo and stored at 4 °C.
(10) For the previous reports on the low stability of commercial subtilisins in THF, see: (a) Fernandes, J. F. A.; Halling, P. J. *Biotechnol. Prog.* **2002**, *18*, 1455. (b) Martinez, S. G.; Alvira, E.; Cordero, L. V.; Ferrer, A.; Montanes-Clementet, I.; Barletta, G. *Biotechnol. Prog.* **2002**, *18*, 1462.
(11) It was observed in our previous studies that the racemizations of aliphatic secondary alcohols were about two times slower than those of benzylic alcohols. See ref 4f.

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